IMPAIRED REGULATION OF CORTICOSTERONE LEVELS DURING FASTING IN AGING RATS

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SUMMARY: The concentration of corticosterone in aortal blood of 3-day fasted rats decreases approximately 75% between 2- and 24-months of age. The reduced level of hormone in aging rats is not attributable to an enhanced rate of corticosterone utilization from the blood or to a diminished steroidogenic capacity of the adrenal cortex, but apparently reflects a deficiency in extra-adrenal regulatory mechanisms.

The adaptive increase in hepatic glucokinase activity following administration of glucose is delayed progressively in time of onset as male Sprague-Dawley rats age from 2- to 24-months(1,2). In contrast, the increase in this enzyme activity in response to exogenous insulin is not impaired during the same portion of the lifespan(1). This, as well as other similar results (3-7), is consistent with the possibility that a deficiency in enzyme regulation may not be intrinsic to the liver. Therefore, it was suggested that the availability of hormonal factors which are crucial to the glucokinase adaptation, e.g., insulin and corticosterone, may be diminished in older rats(4,5). The present report indicates that, under experimental conditions that lead to an altered response of glucokinase activity(1), a modification in the regulation of the circulating level of corticosterone may contribute to this manifestation of aging.

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EXPERIMENTAL

Animals- Intact, male rats of the Sprague-Dawley strain were obtained at 2-, 12- and 24-months of age from a special aging rat colony maintained for R.C. Adelman at the Charles River Breeding Laboratories. The rats were caesarian-derived and barrier-maintained under rigorously controlled environmental and genetic conditions, as will be described with pathology and life-span data in separate publications. Prior to experimentation these rats were maintained in our own facility under the following conditions: in air-conditioned rooms at approximately 72°F.; alternating 12-hr periods of light (6 a.m. to 6 p.m.) and dark; fed a pasteurized, sterilized Charles River diet reported to be of constant composition and component source; all rats were housed singly at least 24 hr prior to experimentation; and rat rooms were frequented by caretaker personnel only following appropriate experimentation.

Treatments- All periods of fasting were initiated at approximately 10:00 a.m., after which time rats were allowed free access to tap water. Bilateral adrenalectomies were performed following anesthetization with ether in our laboratory, after which surgically treated rats were allowed free access to an aqueous solution of 1% NaCl in place of tap water. Rats were injected subcutaneously with 5 units of porcine ACTH (20 units/ml in 0.5% phenol; National Drug) per 100g body weight at 10:00 a.m.

Assay- Blood samples were collected at 10:00 a.m., except where noted otherwise, by inserting a needle directly into the aorta approximately 3 min following initial exposure to ether anesthesia. The concentration of corticosterone in serum was determined fluorometrically, according to the method of Kitabchi and Kitchell(8), using corticosterone (Schwarz-Mann) as a standard. Identity and quantitation of corticosterone were confirmed, and possible interference with the assay procedure by fluorescing metabolites was ruled out by means of thin layer chromatographic analysis of serum samples of blood collected from the portal vein following intraperitoneal injection of corticosterone standard(9).

RESULTS

The concentrations of corticosterone in serum of blood collected from the aortas of 3-day fasted rats of different ages are shown in Table IA. As rats age from 2- to 24-months, this hormone level decreases approximately 75% from 42 to 9 µg per 100 ml of serum. As also indicated in Table IA, injection of ACTH to 3-day fasted 12-month old rats increases the aortal blood level of corticosterone to an even higher value than that observed in the 3-day fasted 2-month old rats not treated with ACTH. Since the decreased level of corticosterone already was quite evident at 12-months of

Table I

Age-Dependent Regulation of Corticosterone Levels during Fasting

	Condition	Age (months)	Corticosterone Concentration (µg/100ml serum)
A.	3-day fasted	2	42 ± 3 (23)
		12	14 ± 2 (23)
		24	9 ± 1 (6)
	3-day fasted and treated with ACTH	12	67 ± 3 (6)
B.	time after adrenalectomy of fasting rats:		
	0	2	60 ± 3 (6)
		12	76 ± 4 (6)
	3 hours	2	28 ± 3 (5)
		12	39 ± 3 (6)
	1 day	2	41 ± 4 (6)
		12	30 ± 2 (6)
	2 days	2	24 ± 2 (6)
		12	7 ± 1 (6)

A) Fasting conditions, ACTH treatment and the corticosterone assay procedure are described in the EXPERIMENTAL section. ACTH-treated rats were sampled 40 min following injection at 10:00 a.m. B) Following a 24-hr fast beginning at 10:00 a.m., 2- and 12-month old rats were anesthetized with ether for 40 and 50 min., respectively, in order to elevate the endogenous circulating level of corticosterone. Rats then were adrenalectomized bilaterally and maintained on aqueous 1% NaCl without food for the duration of the experiment. At the indicated times following adrenalectomy, hormone levels were measured in aortal blood, as described in the EXPERIMENTAL section. Days 0, 1 and 2 after adrenalectomy correspond to days 1, 2 and 3 fasting.

Each value represents the mean ± standard error for the number of rats indicated in parentheses.

age, only these animals were treated with ACTH in order to conserve the exceedingly more expensive 2-year old rats. Although fasting may increase the rate of corticosterone utilization from blood to a greater extent in older rats, the differences are small, as indicated in Table IB.

DISCUSSION

Unstimulated circulating levels of corticosterone in rats aged 2- to 24-months are approximately 5 to 10 μg per 100 ml of serum under environmental conditions similar to those described above(9,10). Furthermore, the amount of corticosterone associated with serum proteins remains the same as rats age from 2- to 24-months, only slightly less than 100%(9).

The impaired ability to increase circulating levels of corticosterone when rats of increasing age are subjected to the stress of starvation probably represents a specific lesion of aging, that is not related to growth or obesity(9). This may be of extreme importance to age-associated impairments in the regulation of glucocorticoid-sensitive enzymes in fasted rats. For example, the age-dependent adaptation of hepatic glucokinase activity following administration of glucose was observed in 3-day fasted rats(1). The progressively increasing delay in the time required to initiate this enzyme adaptation in rats aged 2-, 12- and 24-months is accompanied by the progressively reduced ability of 3-day fasted rats to increase the circulating concentration of corticosterone; i.e., 400% at 2-months, 50% at 12-months, and not at all at 24-months.

The age-dependent impairment in regulation of corticosterone levels probably cannot be ascribed to an enhanced rate of hormone utilization in older fasted rats(Table IB). Therefore, the inability to increase corticosterone levels probably is the consequence of an alteration in the control of hormone production. The steroidogenic capacity of the adrenal cortex *in vivo* in response to exogenous ACTH probably is not impaired in 3-day fasted aging rats (Table IA). Therefore, the aging lesion may relate to regulation of the availability and/or effectiveness of endogenous ACTH.

A previous report of hypoadrenalism *in vivo* accompanied by adequate adrenal responsiveness to exogenous ACTH is the apparent consequence of a circulating pool of inactive endogenous ACTH(11). Furthermore, a previously reported increase in corticosterone levels during fasting of young adult rats is nearly abolished by thyroidectomy(12). The conceivable importance of age-dependent changes either in the availability or action of thyroid hormone(13) or in the accumulation of an inhibitor of thyroxine action(14) remains to be determined. Finally, in view of the recent suggestion that modification of circadian activity rhythms may be of importance to aging (15), the possibility that starvation modulates the circadian rhythm of corticosterone levels differently at different ages also warrants examination.

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